IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application: Hoon HAN *et al.*	
U.S. Application No.: 10/579,070	Art Unit: 1633
Confirmation No. 3303	Examiner: Fereydoun G. SAJJADI
Filed: May 11, 2006	Atty. Docket No.: 36470-231114
Title: METHOD OF ISOLATING AND CULTURING MESENCHYMAL STEM CELL DERIVED FROM UMBILICAL CORD BLOOD	Customer No. 26694 PATENT TRADEMARK OFFICE

DECLARATION OF HOON HAN UNDER 37 C.F.R § 1.132

Commissioner for Patents
Post Office Box 1450
Alexandria, Virginia 22313-1450

Madam:

I, Hoon HAN, M.D., Ph.D., do declare as follows:

1. I am a named inventor in the above identified patent application.

2. I have earned a degree of MD from Catholic University of Korea. My studies involved an exchange professor in UCLA.

3. My current position is CEO at Histostem Corporation. My position involves Director of R&D Center of Histostem Corporation. I have also been a chairman of The Korean Society for Biomaterials.

4. The invention claimed in the present application is for the isolation and culturing of mesenchymal stem cells (MSCs) from umbilical cord blood (UCB), which is a poor source of MSCs. The culturing step uses an αMEM medium containing the growth factors: Stem Cell Factor, GM-CSF (granulocyte-macrophage colony-stimulating

factor), G-CSF (granulocyte colony-stimulating factor), IL-3 (interleukin-3), and IL-6 (interleukin-6). As set forth in the application, conventional methods of culturing MSCs, including MSCs from UBC utilize a culture medium that does not contain the growth factors. The Erices reference identified by the Examiner is an example of this conventional method of growing MSCs from bone marrow.

5. It is known that isolation of MSCs from bone marrow according to Erices's method is not difficult. However, isolation of MSCs from UCB has been very difficult both technically and theoretically before the subject invention. Until the year 2003, it is known that umbilical cord blood contains plenty of hematopoietic stem cells, but rarely contains mesenchymal stem cells, as described in, for example, *STEM CELLS* 2003; 21:105-110; *British Journal of Haematology*, 2003, 121, 368-374; and *Hematologica* 2001; 86:1099-1100. Before the subject invention, UCB has been used to provide hematopoietic stem cells only, as shown for example in Nishikawa *et al*. There has been little interest in UCB except for isolation and expansion of hematopoietic stem cells therefrom. Despite such situation, I have studied UCB steadily from the 1990s, and I have 14 papers in SCI only regarding MSCs. One of the advantages of using UCB as a source of MSCs is that isolating MSCs from UCB has less adverse effect compared to that from bone marrow.

6. The culturing of MSCs from UCB is very different from the culturing of MSCs from bone marrow or a commercial sample of MSCs. This is because there are very few MSCs present in UCB and it would not be expected to obtain a significant number of viable MSCs from UCB. Because the source and viability of MSCs is very different, one would not expect or predict that using a growth medium for MSCs from bone marrow or a commercial source would improve the growth and isolation of MSCs from UCB.

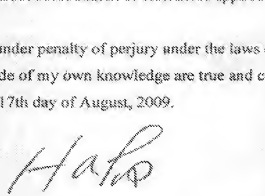
7. As shown in Example 3 and Table 2 of the present application, the use of an αMEM medium containing Stem Cell Factor, GM-CSF (granulocyte-macrophage colony-stimulating factor), G-CSF (granulocyte colony-stimulating factor), IL-3 (interleukin-3), and IL-6 (interleukin-6) unexpectedly provides a 49 fold increase in the isolation of MSCs from UCB. In Example 3, 1 unit of UCB means the amount of UCB obtained from 1 woman after parturition and 50 units of UCB have been obtained from 50

different women after parturition. From these 50 units of UCB is obtained 1 unit of MSCs using the conventional method without growth factors, which is a 2 % success rate (1 unit out of 50 units). In contrast, as also shown in Example 3, by use of the inventive method one obtains 49 units of MSCs using the conventional method without growth factors, which is a 98 % success rate (49 units out of 50 units). For the reasons stated above, that is the very different nature of the sources, this result is unexpected from the addition of the identified growth factors.

8. I also understand that the examiner believes that paragraph [0076] of US Patent Application Publication No. 2004/0235160 (Nishikawa) describes the culturing of a mixture of MSCs and Hematopoietic Stem Cells (HSCs) obtained from Example 5 of Nishikawa using a medium that includes a number of growth factors. This is not true. Paragraph [0076] describes the further culturing of CD34-positive HSCs that are obtained from the co-culturing process of Example 5. In example 5, the MSCs are used as feeder cells in growing HSCs and Nishikawa does not show that the MSCs grow. The only types of cells shown to grow in the process described in paragraph [0076] of Nishikawa are CD34-positive HSCs. Thus, Nishikawa does not disclose the use of a culture medium containing the recited growth factors for culturing MSCs.

9. For the reasons stated above, the results obtained using the method claimed in the present application are unexpectedly superior to the results a person skilled in the art would expect from combination of references applied by the examiner.

I declare under penalty of perjury under the laws of the United States of America all statements made of my own knowledge are true and correct.
Executed on this 17th day of August, 2009.



Hoon HAN